Familial Neuroblastoma: A Three-Generation Pedigree and a Further Association With Hirschsprung Disease

John M. Maris, MD, Jane Chatten, MD, Anna T. Meadows, MD, Jaclyn A. Biegel, PhD, and Garrett M. Brodeur, MD

Like the other embryonal cancers of childhood, neuroblastoma occasionally occurs within families. We now provide an update on a nuclear family in which seven individuals are affected with neuroblastoma, inherited in an autosomal dominant fashion over three generations. In addition, two of these individuals are also affected with Hirschsprung disease. This family may lend insight into the molecular pathogenesis of familial neuroblastoma.

Key words: neuroblastoma, genetic predisposition, pediatric malignancy, embryonal cancer

INTRODUCTION

Neuroblastoma is a relatively common pediatric malignancy derived from the postganglionic sympathetic nervous system [1]. Like retinoblastoma and Wilms' tumor, it is an embryonal cancer, presumably originating from a neuroblast that has not completed the process of neuronal differentiation. The biologic behavior of this tumor is highly variable and can range between spontaneous regression to relentless progression.

Familial clustering of neuroblastoma patients have been reported [2–16]. Indeed, hereditary predisposition in a subset of patients with neuroblastoma is predicted by the Knudson two hit hypothesis of tumorigenesis [17]. A family reported by one of us (J.C.) in 1967 remains the most extensive pedigree of familial neuroblastoma [2]. Identification of two additional affected individuals within this family, one a half sibling and the other in a third generation, as well as an association with Hirschsprung disease in two of the affected members, offers further insight into hereditary predisposition to neuroblastoma.

MATERIALS AND METHODS

All affected individuals were treated at the Children's Hospital of Philadelphia (CHOP). Clinical information was obtained either through interview or medical record chart review. Most of the unaffected individuals were examined by one of us (J.C., A.T.M., J.M.M.) or a reliable local physician.

A protocol for specimen collection and molecular genetic analysis of inherited neuroblastoma has been approved by the CHOP Institutional Review Board. This family is included in an ongoing project to localize a familial neuroblastoma predisposition gene by linkage analysis. DNA was extracted from either peripheral blood lymphocytes or paraffin-embedded tissue according to standard methodology [18]. A partial constitutional genotype was derived by the analysis of a panel of polymerase chain reaction (PCR)–based polymorphisms located on the distal short arm of chromosome 1, as previously described [19,20]. Briefly, constitutional DNA from each individual was amplified by PCR using primers end labeled with ^{33}P-γdATP. A panel of 1p36 simple tandem repeat polymorphisms *D1S243, D1S468, D1S214, D1S1646, D1S160, D1S548, D1S489,* and *D1S507* (Genome Database) have been assembled for linkage analysis and were used to amplify the DNA of individuals I-2, II-4, II-5, III-6, III-8, III-11, III-12, IV-4, and IV-5. The resulting PCR products were resolved in 8% polyacrylamide/7 M urea, run in parallel with a known sequencing reaction, and visualized by autoradiography at room temperature for 2–4 hours.

Genotype analysis and assignment of haplotypes was performed with the FASTLINK program.

PEDIGREE INFORMATION

The pedigree is shown in Figure 1. The case histories of the five full siblings in the third generation were detailed in our initial report. Briefly, four of the five children

From the Division of Oncology (J.M.M., A.T.M., G.M.B.), Department of Pathology (J.C.), and Division of Human Genetics and Molecular Biology (J.A.B.), The Children's Hospital of Philadelphia and the Department of Pediatrics, The University of Pennsylvania, Philadelphia, Pennsylvania.

Received August 23, 1995; accepted January 5, 1996.

Address reprint requests to John M. Maris, MD, Division of Oncology, The Children's Hospital of Philadelphia, The Abramson Pediatric Research Building, Suite 902, 324 South 34th Street, Philadelphia, PA 19104.

had histopathologically confirmed neuroblastoma. Multifocal primary tumors were documented in patients III-9, III-10, and III-11. All children were diagnosed before 15 months of age. Patient III-6 had neuroblastoma in situ detected as an incidental finding during an autopsy following fatal total aganglionosis. The unaffected individual (III-8) remains disease free and currently is alive and well, as are his three children, ages 8–11 with no clinical evidence of Hirschsprung disease or occult neuroblastoma. These children are routinely assessed by detailed physical examination, but have not had screening of urinary catecholamines. The only affected child alive at the time of the initial report (III-11) has never had a recurrence and currently has normal urinary catecholamines. The diagnosis of a related neural tumor in the mother (II-4) of these children was retrospective. She was demonstrated to have a presumptive thoracic ganglioneuroma on the basis of a retrocardiac density on chest roentenography and elevated urinary catecholamines [21].

Since the time of the original report, I-1 and II-2 have died from coronary artery disease. The remaining six individuals in the second generation continue to have no clinical evidence of occult neuroblastoma or Hirschsprung disease. Furthermore, the 25 offspring of these individuals are reportedly healthy and free of symptoms that would suggest either of these two conditions.

Patient III-6 was diagnosed with disseminated neuroblastoma in 1961 when she was referred to the Children's Hospital of Philadelphia at the age of 4 years 4 months. She was an adopted girl who was previously healthy, and the biologic mother was not known to us at the time. The diagnosis of neuroblastoma with bone and bone marrow dissemination was established by biopsy of a left adrenal mass and the bone marrow. Recent review confirmed the presence of neuroblastoma with unfavorable histopathologic features by the Shimada classification [22]. Despite treatment with the antimetabolite 2-deoxy-5-fluorouridine the primary and metastatic lesions rapidly increased in size. She died at the age of 4 years 6 months. An autopsy was not performed, and there was no history consistent with Hirschsprung disease upon chart review.

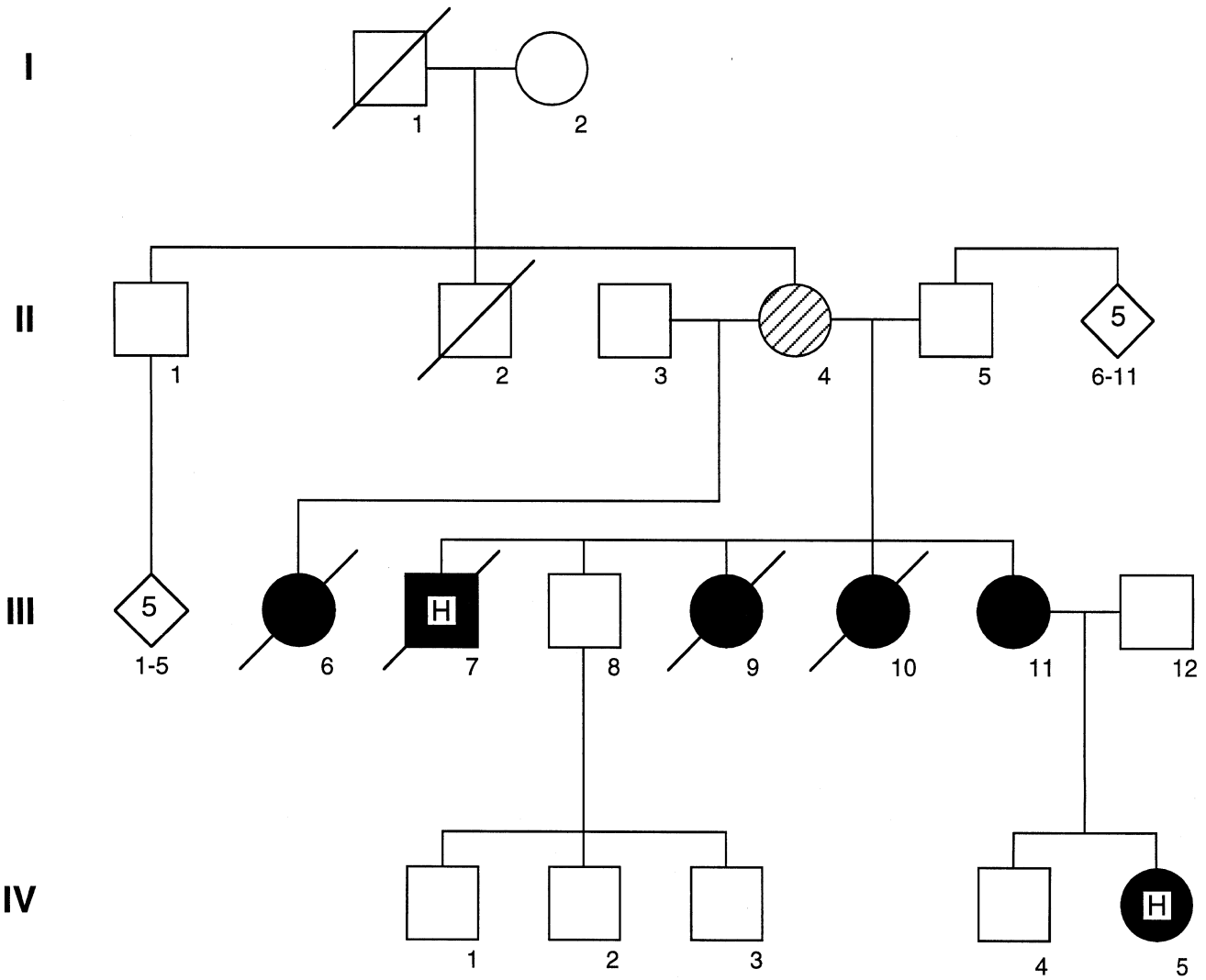
We only recently learned that this patient was the first child of the asymptomatically affected mother (II-4), and, therefore, a half-sibling to the originally reported five children. Supportive molecular genetic evidence for maternity was made by analysis of a panel of highly informative di- and tetra-nucleotide repeat polymorphisms located on the distal short arm of chromosome 1. DNA extracted from paraffin-embedded tissue from patient III-6 was compared to DNA extracted from peripheral blood lymphocytes of each of the surviving individuals within the four generations (Fig. 2). Individual III-6 had a genotype consistent with inheritance of a haplotype from the mother (II-4), but not the known father (II-5). The sharing of an identical haplotype at 1p36 is strong evidence to support the historical information regarding maternity (Fig. 2).

Patient II-11 is presently alive and well, with normal urinary catecholamines. She has two children, currently 5 and $3\frac{1}{2}$ years of age, from a non-consanguineous marriage. Her husband (III-12) has no historical or physical examination evidence of Hirschsprung disease, nor do his three siblings. The older boy (IV-4) is healthy. Because of his family history, he had repeated measurements of urinary catecholamines and abdominal ultrasonography examinations until the age of 2. These were normal, and he currently has a normal physical examination with no suggestion of occult neuroblastoma or Hirschsprung disease.

Patient IV-5 came to medical attention at 3 weeks of age because of severe diarrhea resulting in hypovolemic shock. A diagnosis of Hirschsprung disease was established on rectal biopsy. Prior to surgical correction and because of her family history, urinary catecholamines were measured and found to be elevated for her age. A computed tomographic scan revealed multifocal neuroblastoma involving the right adrenal and intra-abdominal paraspinal region. At 1 month of age she underwent a right adrenalectomy and removal of the paraspinal lesion, as well as a transverse loop colostomy for the aganglionosis (transition zone at the mid-transverse colon) and a Ladd procedure (for the incidental finding of an intestinal malrotation). The tumors had a favorable histopathologic pattern [22], and the *MYCN* proto-oncogene was not amplified.

After 2 months of observation the tumor recurred within the celiac axis. She was initially treated with oral cyclophosphamide, but was switched to multiagent therapy with cisplatin, doxorubicin, etoposide, and cyclophosphamide because of tumor progression. After 3 months of this therapy, locally progressive disease was evident, and she was switched to vincristine, ifosphamide, and etoposide for two cycles. Her disease stabilized, and she was initially maintained with vincristine and cis-retinoic acid. However, locally progressive disease caused ureteral obstruction and hydronephrosis. Surgical debulking at the age of 21 months relieved the obstruction and histopathologic examination demonstrated differentiating neuroblastoma.

Despite withdrawal of chemotherapy shortly after her debulking procedure, her disease status has not changed. She continues to have a large, midline tumor that is unresectable but without evidence of growth or metastatic spread. Urinary catecholamines remain elevated. Interestingly, her tumor secretes high quantities of insulin-like growth factor II that caused hypoglycemia severe enough to induce seizures, which are presently controlled with pharmacologic doses of growth hormone [23]. A needle



KEY

= GANGLIONEUROMA

= NEUROBLASTOMA

H = HIRSCHSPRUNG DISEASE

GENOTYPES	II-4	II-5	III-6	III-8	III-11
D1S243	1 4	11 11	2 4	11 1	11 1
D1S468	4 2	1 5	1 2	1 4	1 4
D1S214	3 1	1 2	4 1	1 3	1 3
D1S1646	4 5	1 4	3 5	4 5	4 5
D1S160	5 5	3 6	5 5	3 5	3 5
D1S548	6 6	5 7	6 6	7 6	7 6
D1S489	5 5	2 5	2 5	2 5	2 5
D1S507	2 5	2 6	7 5	6 2	6 5

Fig. 1. A family segregating for neuroblastoma and Hirschsprung disease through three generations. All unaffected individuals were clinically evaluated, but only IV-4 has had screening urinary catecholamines. Inferred haplotypes at chromosome 1p36 for selected individuals are listed, with the shared haplotype between II-4 and III-6 boxed.

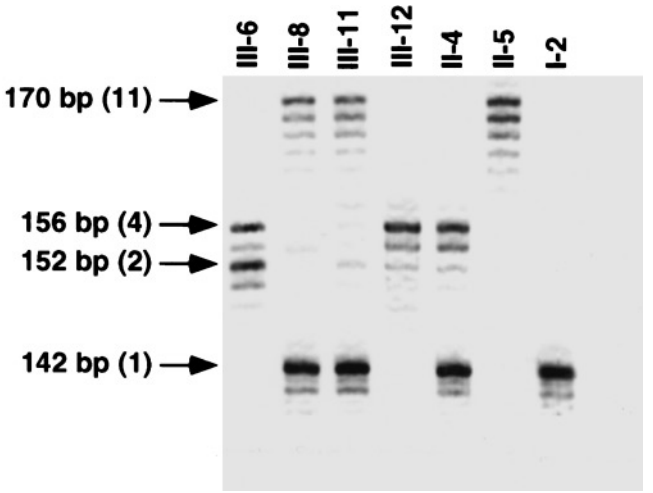


Fig. 2. Representative allelotypes of selected family members at *D1S243.* Allele sizes are indicated as number of base pairs (bp) along with the allele number assigned by the genome database. The 152-bp allele 2 is unique to patient III-6, and there are no shared alleles, and therefore no possibility of blood relation, between individuals II-5 (11,11) and III-6 (2,4). III-6 shares the 156 bp allele 4 with II-4, whereas III-8 and III-11 inherit the 142 bp allele 1 from II-4 and the 170 bp allele 11 from II-5.

biopsy of the tumor obtained during a recent revision of her colostomy showed ganglioneuroma.

DISCUSSION

Hereditary predisposition to the pediatric embryonal cancers is predicted by the two-hit model of tumorigenesis proposed by Knudson and Strong [17]. Whereas two somatically acquired mutations in a tumor suppressor gene would be necessary for tumor formation in sporadic cases, familial predisposition would occur due to a germline mutation in one allele followed by a somatically acquired inactivation of the remaining allele in the target tissue. It follows that familial cases would usually bear the hallmarks of an earlier age of onset and multifocality. This is clear in the case of retinoblastoma, in which a single tumor suppressor gene on chromosome band 13q14 is involved in all children with hereditary or sporadic disease. Germline mutations in the *RB1* gene predispose a child to the acquisition of multiple, bilateral tumors at an earlier age of onset than those children with two somatically acquired mutations [24,25]. However, recent evidence suggests that the molecular genetic pathogenesis of neuroblastoma may be more complex, and more than one tumor suppressor gene is probably involved [26]. It is not yet clear if the familial cases of neuroblastoma share genetic homogeneity or if different susceptibility genes are mutated in different families.

Neuroblastoma has been associated with Hirschsprung disease, usually as a sporadic occurrence [27,28]. There is one report of a family segregating for both disorders [15]. The occurrence of neuroblastoma and Hirschsprung disease together, along with other congenital abnormalities such as central hypoventilation, suggests a common molecular genetic event manifesting as a neurocristopathy. In the family reported here, there is no history of Hirschsprung disease in the first-degree relatives nor is there consanguinity. This suggests that Hirschsprung disease and neuroblastoma are co-segregating and that one heritable genetic event may explain both conditions. Recent studies implicating the *RET* proto-oncogene in autosomal dominant Hirschsprung disease [29] and its postulated involvement in neuronal differentiation [30] and perhaps neuroblastoma [31] may provide a common link between these diverse clinical entities.

In summary, we report on the status of a three-generation pedigree segregating for neuroblastoma inherited in an autosomal dominant fashion and associated with Hirschsprung disease. This family may lend insight into the complicated molecular genetics of neuroblastoma. We have begun a multi-institutional effort to ascertain families with more than one first-degree relative affected with neuroblastoma. Locus-specific linkage analysis to identify a neuroblastoma susceptibility gene has begun. Additional families to aid in our search would be welcome.

ACKNOWLEDGMENTS

The authors thank Susan Kyemba for her technical assistance with the DNA technologies and Dr. Timothy Rebbeck for his advice and assistance with the genotyping. We would also like to gratefully acknowledge the members of this family for their enthusiastic cooperation with our research efforts.

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